Environmental Monitoring Review and Trending: Two Case Studies on the Importance of Assessing Sampling and Results

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Case Study 1: Evaluating water system trends

Introduction

A risk assessment was performed on a water system to assess the risks associated with sampling locations and evaluate longer-term data trends of alert/action and maintenance events. Through data analysis of the existing sampling and trend data over time, we were able to determine the root cause of annual TOC out-of-specification results of the water system.

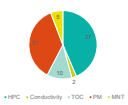
Data Analysis Methodology

1. First, overall data was reviewed and graphed in figure 1.1 to determine potential trends of alert/action and maintenance events.

- Heterotrophic plate count testing (HPC) represented the highest number of alert/action levels observed with a count of 37.
- Total organic carbon (TOC) had the second highest amount of alert/action levels with a count of ten (10).

NOTE: Maintenance events (i.e., preventative – PM, and on demand - MNT) was not considered for trending as they are required at regular standard intervals and/ or as needed.

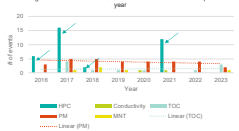
Figure 1.1: Number of alert/ action and maintenance events from 2016-2023



2. Next, data was analyzed per year to determine if annual trends were observed among alert/action and maintenance events (see figure 1.2).

- HPC spikes observed in 2016, 2017, 2018 and 2021 require further investigation (see section 3).
- PM events are at standard intervals and do not change year-over-year.
- Although the number of TOC alert/action events were low, a trend was observed annually requiring further investigation (see section 4).

Figure 1.2: Number of alert/ action and maintenance events per year



3. Heterotrophic plate count (HPC) trends observed in 2016 – 2018 and 2021 were investigated further.

- HPC trends from 2016 to 2018 revealed 96% of activities were from system ports with action levels defined. It is normal for system ports to result in significant growth as port locations were prior to purification. Acceptance criteria of system ports was increased in 2019 and ultimately changed to "for information only" in 2022.
- HPC trends in 2021 revealed 85% of activities were from a single USP port where tubing was left in place and used during sampling. Changes were made to use sterile tubing only when needed in 2022.

4. Data was analyzed per quarter to drill down further on the annual TOC trend observed (see figure 1.3).

- All TOC alert/action events were observed in Q2, along with the greatest number of PM activities, increased levels of HPC and conductivity alert/action events.
- The greatest number of HPC alert/action events were observed in Q1, followed by Q4 and Q2. Review of this data carried over from annual trend results previously discussed in section 3.

Figure 1.3: Number of alert/ action and maintenance events per quarter from 2016-2023

5. Data was filtered and analyzed by quarter two to see if there was a relationship between TOC alert/action and maintenance events (see figure 1.4) .

- Increased TOC levels were reported on the same day (and up to two days after) preventative maintenance (PM) activities were performed except for 2019 and 2021.
- All PMs performed during this time included sanitization of the water system (using Minncare®).

Figure: 1.4: Alert/action and maintenance events filtered by quarter two (Q2) from 2016-2023

Date	Activity	Result/ Detail	Spec
03-May-17	PM	Sanitization performed during annual PM.	No residual Minncare detected via strips, prior to release
	TOC	275 ppb	USP <500 ppb; alert 175 ppb
	TOC	266 ppb	USP <500 ppb; alert 175 ppb
	TOC	268 ppb	USP <500 ppb; alert 175 ppb
	TOC	182 ppb	USP <500 ppb; alert 175 ppb
16-Apr-18	PM	Sanitization performed during annual PM.	No residual Minncare detected via strips, prior to release
	TOC	1510 ppb	USP < 500 ppb
23-Apr-18	HPC	10 CFU/ mL	USP <100 CFU/ mL; alert 10 CFU/ mL
22-Apr-19	PM	Sanitization performed during annual PM.	No residual Minncare detected via strips, prior to release
29-Apr-20	PM	Sanitization performed during annual PM.	No residual Minncare detected via strips, prior to release
	Conductivity	1.9 uS/ cm	Conductivity Alert Limit 1.9 (uS/cm)
	TOC	465 ppb	USP < 500 ppb; action 425 ppb
23-Apr-21	PM	Sanitization performed during annual PM.	No residual Minncare detected via strips, prior to release
30-Apr-21	HPC	16 CFU/ mL	USP <100 CFU/ mL; alert 10 CFU/ mL
21-Apr-22	PM	Sanitization performed during annual PM.	No residual Minncare detected via strips, prior to release
22-Apr-22	TOC	794 ppb	USP < 500 ppb
12-Apr-23	PM	Sanitization performed during annual PM.	No residual Minncare detected via strips, prior to release
	TOC	1630 ppb	USP < 500 ppb
13-Apr-23	TOC	666 ppb	USP < 500 ppb
14-Apr-23	TOC	882 ppb	USP < 500 ppb

6. Interviews were conducted with cross-functional team members to understand: (a) Why alert/action events for TOC were not observed in 2019 and 2021, (b) Why the system was released for use, yet failed TOC, (c) Why this annual TOC trend was not observed on the alternate water system and (d) Why this trend was not captured during periodic review of the water system.

- (a) System operators explained that increased TOC results had been observed historically, post-sanitization. In 2019 and 2021, the system was not released until passing results were obtained on April 25, 2019. Therefore, results were not captured as an alert/ action event and do not show up in the data.
- (b) The system vendor explained that the system is released after residual Minncare® is no longer detected by test strips. The facility manager pointed out that the limit of detection of the TOC test is 1000 times greater (parts-per-billion) than residual Minncare® test strips (parts-per-million).
- (c) The system vendor also explained that this water system is smaller than the alternate system and takes longer to polish the water and remove impurities post-sanitization.
- (d) Periodic review of the water system is performed annually. Trends are evaluated over a 12-month period.

Conclusion

Analyzing existing sampling and trending data of a water system over time was able to explain out-of-specification TOC results. To avoid future TOC alert/action levels, updates were made to the utility system procedure requiring passing TOC results prior to release of the system post-sanitization. Risk assessments are performed throughout the lifecycle to review trends greater than 12-months, as applicable. The remaining data showed that the system was in a state-of-control and justified reduction of sampling ports for routine monitoring.

Data analysis is like a puzzle. Each piece is a small part of the bigger picture. All the "pieces" combined tell a story.

Revealing the story the data tells is not always cut and dry. Analyzing data requires looking at it from every angle to see where it fits into the big picture.

Case Study 2: Investigating contamination in a cleanroom

Introduction

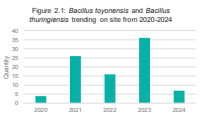
Bacillus contamination was detected on personnel monitoring plates in the sterility suite. However, the contaminant was not captured during routine environmental monitoring of the cleanroom. An investigation was performed to examine all possible areas where contamination could enter the cleanroom as well as why this contamination was not being identified during routine monitoring. Through data trending, experimental testing, and additional sampling, potential sources of contamination were identified, and mitigations were put into place.

Investigation Process

1. First, trending was performed to determine if the identified organisms had been found previously on site (see figure 2.1).

- *Bacillus toyonensis* and *Bacillus thuringiensis* are found in soil and are often identified in uncontrolled spaces.

Figure 2.1: *Bacillus toyonensis* and *Bacillus thuringiensis* trending on site from 2020-2024



4. A second experiment was designed to determine if paper autoclave pouches were permeable when wiped with multiple disinfectants during the staging process.

- Paper autoclave pouches containing sterile forceps were set up to test the following two methods (a and b) against three disinfectants: 70% IPA, Vesta-Syde®, and 20% Bleach (see figure 2.3).
  a. Inoculate paper side of autoclave pouch with organism first and allow to dry; wipe with disinfectant; allow 10-minute contact time.
  b. Wipe paper side of autoclave pouch with disinfectant first and inoculate with organism while saturated; allow 10-minute contact time.
- Pouches were opened aseptically, and "sterile" forceps were transferred to TSB. Forceps were incubated for 7 days at 30-35°C.
- Growth was obtained in samples that were inoculated with organism first and then wiped with either 70% IPA or Vesta-Syde®.

2. Next, an investigation was performed with cross-functional team members to determine how these organisms were getting into the ISO 5 space?

Initial Question	Information from Investigation	What needs further Investigation
Are analysts being aseptic? →	Yes, gowning qualifications and historical data show analysts have proper aseptic technique. →	If analysts are aseptic and gowning properly, what other factors may be contributing to contamination?
Is cleaning sufficient? →	Cleaning procedure in place with rotational disinfectants for routine room cleanings as well as staging of materials. Transfer carts are not included in routine cleaning. →	Are carts bringing contamination into the cleanroom?
Is environmental monitoring capturing enough data? →	There were no excursions found during review. However, routine monitoring is not performed on the carts used for media transfer. →	Are the highest risk areas being sampled?
What are possible contaminated surfaces? →	Sterilization pouches used to autoclave utensils are paper and may be permeable when wet. →	Could the paper pouches allow organism to permeate when wet?

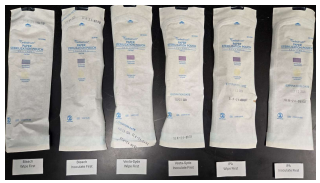


Figure 2.3: Experimental layout of autoclave pouches

3. An experiment was designed to determine if carts were bringing contamination into the cleanroom.

- Carts are only cleaned with 70% IPA to enter the ISO 8 environment. 70% IPA is not effective against spore-forming organisms.
- Surface samples were taken from carts inside of the cleanroom and in the main laboratory space, prior to being staged, for information only. See figure 2.2 for example of organisms recovered from surface of carts.
- *Bacillus toyonensis* and *Bacillus thuringiensis* were both identified on surfaces and wheels of carts inside of the cleanroom and in the main laboratory space.

Figure 2.2: Example of organisms recovered from surface of carts.

Conclusion

Data trending, experimental testing, and additional sampling were used to identify potential sources of *Bacillus* contamination of personnel in a sterility suite. *Bacillus* species were previously found onsite on transfer carts and in uncontrolled environments. Transfer carts and paper autoclave pouches were determined to be likely sources of contamination due to current cleaning and/ or staging practices. Mitigations were put into place to reduce the likelihood of reoccurrence: new carts were purchased and designated for cleanroom use only, an engineering study was performed to allow items to be double bagged for cleanroom use, and 20% bleach is now used to stage items into the cleanroom. Environmental monitoring is being reassessed to ensure all critical control points are sampled during routine monitoring.

****Highlights****

- When reviewing trends, it is important to collect data from ALL available sources impacting the system, location, or process under investigation, including, but not limited to quality events and maintenance activities.
- Gather and review as much data as is readily available. Twelve months is not enough when reviewing data from long standing systems.
- When analyzing data, it is important to start big and drill down on each trend observed to determine the root cause of quality events.
- Assemble and collaborate with a cross-functional team of subject matter experts, operators, facility managers and vendors to fully understand data trends.